The Effect of Dosing and Cryopreservation on Efficacy and Safety of a Novel Cell Therapy for Degenerative Disc Disease Using a Porcine Model: Sub-Acute and Chronic Timepoints

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Introduction

- Degenerative disc disease (DDD) is a primary cause of low back pain. There is a great need for novel treatments for DDD.
- Our lab isolates progenitor cells from human disc tissue and creates a therapeutic cell population known as '*discogenic cells*'. These cells are multipotent, express a unique profile of surface markers, do not form tumors, and produce proteoglycan, collagen and anti-inflammatory cytokines.
- To assess safety and possible clinical efficacy, we injected discogenic cells mixed with hyaluronic acid gel into injured discs of 9 Gottingen minipigs™, building upon previous research in rabbits. Cell dose and cell form (fresh or cryopreserved) were assessed after 24 hours and 4 weeks. We evaluated disc height, histology, cell persistence, MRI, 22 hematological parameters, 19 clinical chemistry panel parameters, body weight, and clinical behavior.
- This study will help to identify the optimal dosing to be translated to human clinical trials, and also will help to finalize the formulation intended for human use.

Methods

- Using a previously validated model, three lumbar discs of 9 Gottingen minipigs™ were injured with 3 needle punctures (*note*: study performed after approval of private IACUC).
- After four weeks, each animal received either scaffold (1% hyaluronic acid gel) with 10,000 cryopreserved (CP) cells, 100,000 CP cells, 1,000,000 CP cells or 1,000,000 fresh cells, or scaffold alone, or a sham injection (n=3/animal). The final two animals were either treated with 100,000 CP cells that were loaded with fluorescent calcein or subjected to a sham injection. Needle placement at delivery was confirmed using multi-plane fluoroscopy (**Figure 1A**).
- Disc height was measured by 18 boney landmarks using x-ray images and normalized to week 0 values, resulting in a disc height index (DHI) that was then used to calculate the change in disc height from week 4 to week 8.
- *Ex vivo* MRI images were acquired at the end of the study using a Varian 7T DirectDrive MRI spectrometer.
- Spines harvested 24 hours and 4 weeks after injection were processed for sagittal histology after paraffin embedding.
- Blood was collected from all animals prior to injury, 3-5 days after injury, and 3-5 days after dose delivery and analyzed for 22 hematological parameters and 19 clinical chemistry panel parameters. Clinical behavior and body weight was also noted.

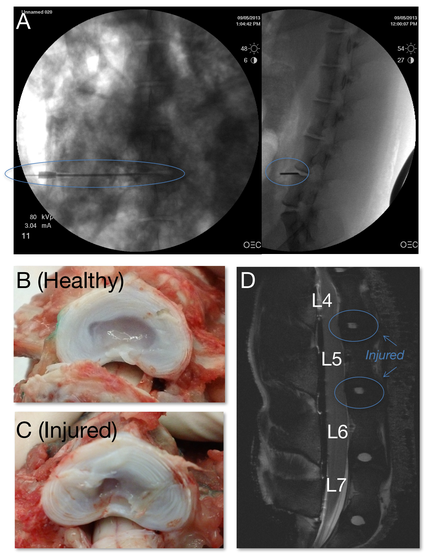


Figure 1: (A) Dual-plane fluoroscopy used to ensure proper needle placement (blue circles) during dose delivery. Gross morphology of (B) healthy pig disc and (C) injured disc after 1 month. (D) T2-weighted MRI image shows darkening of disc after injury (blue circles).

Results

- By aspirating nucleus pulposus from the disc, significant changes occurred that mimic human DDD by the 4 week time point, with changes to gross morphology (**Figure 1B-C**) and darkening of T2-weighted MRI compared to healthy adjacent discs (**Figure 1D**).
- One day after delivery, the human discogenic cells were found in high densities via calcein imaging (**Figure 2A**), showing that the xenograft were not rejected. Also, histology (H&E) did not reveal any inflammation at this sub-acute timepoint, as demonstrated in the transition zone (**Figure 2B**) and in the NP (**Figure 2C**).

Results (con't)

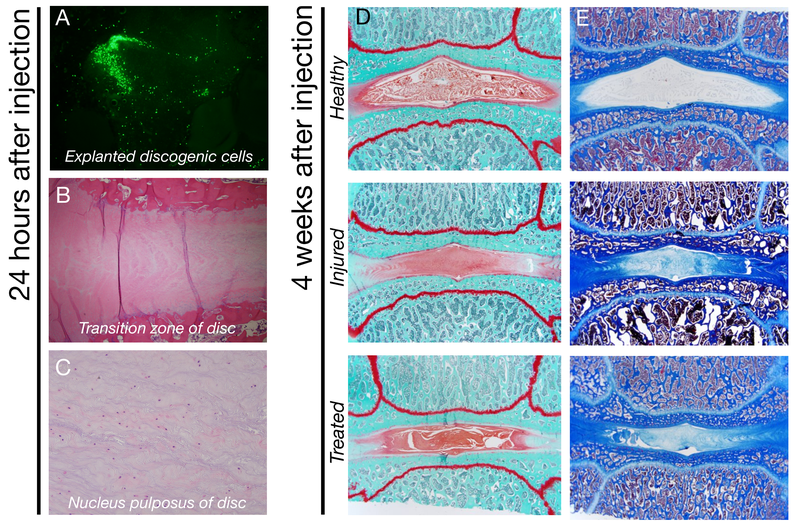
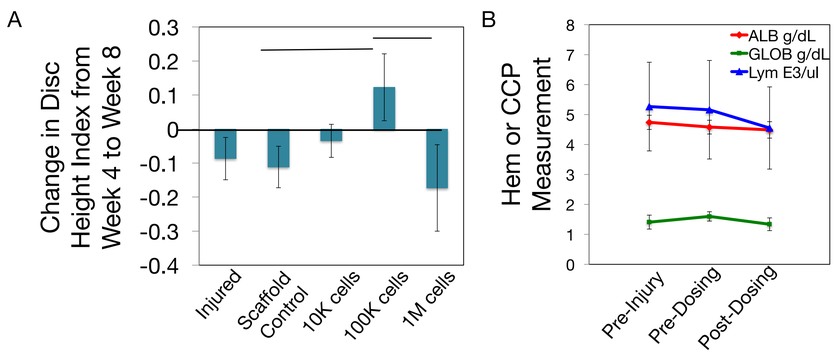


Figure 2: (A) Calcein-labeled cells are found 24 hours after delivery in vivo (4X). H&E staining of (B) transition zone (4X) and (C) nucleus pulposus (20X) shows lack of inflammation 24 hours after delivery. After 1 month, (D) safranin O stain and (E) masson's trichrome stain shows healthy, injured and treated discs.

- Four weeks after delivery, safranin O and masson's trichrome staining showed marked differences between healthy and injured discs, with some improvements noted after treatment as well as an absence of abnormal tissue after delivery of the progenitor cells (**Figure 2D-E**). H&E staining again showed a lack of inflammation (*data not shown*).
- Disc height decreased to 64% of the original height 4 weeks after injury. After treatment, the intermediate cell dose of 100,000 PF cells showed an improvement in disc height between 4 and 8 weeks compared to all other groups ($p < 0.05$) (**Figure 3A**). The disc height was comparable between CP and fresh cell formulations (*data not shown*).
- Body weight was maintained throughout the course of the study (*data not shown*), with no concerning clinical behavior noted. Hematology and clinical chemistry panel results were normal and consistent throughout the course of the study (three parameters are shown in **Figure 3B**).

Figure 3: (A) The 100,000 cell dose improved disc height compared to other cell doses and controls, as measured via x-ray (line indicates difference via 1-way ANOVA, $p < 0.05$). (B) Hematology and clinical chemistry panels show no changes with injury to treatment (ALB = albumin, GLOB = globulin, Lym = lymphocytes).



Conclusions

- A large animal model of DDD was used to test the dosing and formulation of a novel cell therapy for the treatment of degenerative disc disease.
- Cells persisted for at least 24 hours, and no inflammation was noted via histology or through hematology/clinical chemistry panels at sub-acute and chronic timepoints.
- An optimal cryopreserved dose was identified, and will be used in future testing.
- A therapy containing discogenic cells continues to demonstrate efficacy and safety in animal models of DDD, suggesting that future human clinical trials of this therapy is warranted.